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Tradescantia MCN (Micronuclei)-in-Tetrad Mutagen Test-on-Site Monitoring and Further Validation

Western Illinois Univ.
Macomb

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

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TRADESCANTIA MCN-IN-TETRAD MUTAGEN TEST-ON-SITE MONITORING AND FUTHER VALIDATION

By

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Grant Number: 806422010

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16. ABSTRACT

Tradescantia Paludosa (Spiderwort) clone #03, an extensively cultivated experimental plant for cytological studies, has been utilized exclusively for the present investigation. Year-round growth and reproduction of this plant can be maintained in the greenhouse with supplemented artificial light during the short-day season thus facilitating the uninterrupted supply. Taking the advantages of high degree of synchrony and differential sensitivity of the meiotic stages, a short-term bioassay was developed for testing environmental mutagens. This was done by treating the synchronized, sensitive early prophase I meiotic chromosomes and observing the damaged chromosomes in the form of micronuclei in the sychronized early tetrad stage in order to attain the highest efficiency. This test is called Tradescantia Micronucleus (Trad-MCN) Bioassay and has been utilized to screen a group of chemicals in gaseous or liquid forms and monitor the air pollutants at some chosen sites. Of 16 chemicals tested, 8 had relatively higher micronucleus frequencies (0.01 level of significance) than their controls. Of 11 sites monitored, 3 sites showed higher micronucleu frequencies than their controls. According to these results, the Trad-MCN bioassay showed high efficiency and versatility. It is suitable for preliminary screening of environmental pollutants and in situ monitoring.

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FORWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazzards. Careful assessment of the relative risk of existing and new man-made environmental hazzards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminebt and substantial endangerment of their health.

The Laboratory of Environmental Mutagenesis in the Institute for Environmental Management at Western Illinios University has developed a short-term test called Tradescantia Micronucleus Bioassy which can detect the mutagenic of clastogenic effect of liquid or gaseous agents. This report is in response to a grant sponsored by the U.S. Environmental Protection Agency for carrying out in situ monitoring of air pollutants and testing of a group of 16 chemicals some of which are known mutagens or carcinogens.

F. G. Hueter, Ph. D.
Director,
Health Effect Research Laboratory

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

BC	-- Baseline control
C	-- Control
C_d	-- DMSO blank control
C_e	-- ETOH blank control
C_s	-- Sucrose blank control
CMP	-- Chicago municipal parking garage
DEF	-- Diesel exhaust fumes
DEC	-- Diesel exhaust condensate
DMP	-- Decatur municipal parking garage
DMSO	-- Dimethyl sulfoxide
DTS	-- Dixie Truck Stop
ETOH	-- Ethanol
FC	-- Field control
GCI	-- Granite City Industrial site
HC	-- Home control
hr	-- hour
JTC	-- JT cigarette smoke
mM	-- Millimole
MCN	-- Micronuclei
PBS	-- Peoria Bus Stop
PDB	-- Paradichlorobenzene fumes in herbarium
ppm	-- Parts per million
PSR	-- SR public parking garage
R	-- Roentgen unit
SE	-- Standard error
SLW	-- Spring Lake water
T	-- Treated
T_d	-- Treated in DMSO solvent
T_e	-- Treated in ethanol solvent
Trad-MCN	-- Tradescantia Micronucleus
UFO	-- University farm odor from swine house
WRI	-- Wood River Industrial site
WTS	-- Woodhull Truck Stop

SYMBOLS

BaP	-- Benzo(a)pyrene
HC_e	-- Hydrocarbon
NO_x	-- Nitrogen oxides
PbA_c	-- Lead acetate
$\bar{x}$	-- mean
+	-- Positive response
-	-- Negative response
+/-	-- Inconclusive

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The cooperation of Duke Phytotron directed by Dr. H. Hellmers for raising *Tradescantia* plants during the course of diesel emission studies is greatly appreciated.

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SECTION 1

INTRODUCTION

The simple, rapid, inexpensive and reliable eukaryotic bioassays are urgently needed for screening vast numbers of potential hazardous chemicals or wastes created by energy-generating processes from fossil fuel or nuclear fission. Tradescantia Micronucleus (Trad-MCN) bioassay which was developed in 1976 through the studies of mutagenic effects of 1,2-dibromoethane (11,12), meets almost all the requirements as an ideal short-term test. Its efficiency was demonstrated by linear dose-response curve established with varying concentrations of the 1,2-dibromoethane gas. This was further substantiated by the typical dose-response (Fig. 2) to low level X-rays (15). It is suitable for testing gaseous agents and ionizing radiation which can penetrate the buds readily and affect the sensitive chromosomes of early stages of meiosis (15, 18, 19, 21) of the pollen mother cells of Tradescantia. It is also efficient in testing agents of liquid forms which are absorbed through the vascular system of the excised plant stems and dialyzed into the pollen mother cells (12, 13, 14). The high efficiency of this bioassay is attributed to the high degree of synchrony of the early prophase I chromosomes for induction of large number of breaks and these broken pieces of chromosomes can be revealed as micronuclei (MCN) in the synchronized early tetrads (Figs 4 and 5). The scoring of MCN frequency from a large population of tetrads (Fig 5) made the data collection a very simple and easy task. Therefore, this bioassay is ideal for preliminary screening of agents in a battery of short-term bioassays (8).

Since most of the gaseous agents can readily diffuse into the young buds of the inflorescences (17), this test can be used for in situ monitoring purpose similar to the use of Tradescantia stamen hair pink mutation (20).

The present project was designed to further validate this Trad-MCN bioassay under 3 different conditions. These include chemical screening in the laboratory, testing of clastogenicity of diesel engine emissions in collaboration with HERL, US EPA, Research Triangle Park, NC, and in situ monitoring of industrial sites, public parking garages, and bus and truck stops.

SECTION 2

CONCLUSIONS

The *in situ* monitoring of public parking garages and bus and truck stops, was carried out during the period April through September, and the monitoring of industrial sites was done in late fall. Results of 11 sites monitored (some repeatedly monitored), 3 showed significant (0.01 level) higher frequencies of MCN than their controls. One parking garage in Peoria, IL had the MCN frequency 3 - 4 times as high as the controls (Tables 1, 2, and 3).

Diesel exhaust fumes generated by the Nissan automobile at HERL, US EPA, Research Triangle Park, NC were administered to plant cuttings under the 3 different concentrations coupled with 1, 2, or 3 driving cycles (23 minute per cycle). Positive responses were obtained in 2 driving cycles at about 1/23 dilution, and 3 driving cycles at about 1/45 dilution of the fumes. Diesel exhaust condensates from the same source showed no significant positive response at 10, 100, 150, and 200 ppm concentrations and the results were inconclusive (Tables 4 and 5).

Pure gases such as NO_2, SO_2, O_3 and combination of NO_2 plus O_3 were used to treat plant cuttings in the chambers provided by Northrop Services, Inc. Positive results were obtained from NO_2 treatment at 5 ppm concentration when constantly flowed through the chamber for 24 hr, and SO_2 treatment at 1 ppm concentration when constantly flowed through the chamber for 22 hr. Hydrazoic acid gas treatment at 136 ppm concentration by single application in an air-tight chamber showed relative high potency (Table 6).

Of 9 water-soluble agents including ascorbic acid (AA), manganese chloride ($MnCl_2$), sodium bisulfite (NBS), niacin (NIC), sodium nitrite (NNO), propionic acid (PA), lead acetate (pbAc), saccharin (SAC), and zinc chloride ($ZnCl_2$), only 5 (AA, $MnCl_2$, NBS, SAC, $ZnCl_2$) gave significant (0.01 level) higher MCN frequencies than their controls (Table 7).

All 3 water-insoluble agents demonstrated high mutagenicity and toxicity. Benzo(a)pyrene (BaP) showed relatively high potency when sufficient DMSO was used to dissolve the agent fully before mixing with water. Although cyclohexylamine (CHA) and maleic hydrazide (MH) gave positive responses under these preliminary test, further tests are needed to confirm their clastogenicity. The solvent, DMSO, alone at high concentrations 10 - 20% water solution, caused significantly high frequency of MCN as well as physiological damage. Spring Lake water (city reservoir) seemed to be less mutagenic than the chlorinated tapwater which was used as a control in the early spring season (Table 8).

SECTION 3

RECOMMENDATIONS

Based upon the results of this series of studies, Trad-MCN bioassay has been shown to be well-suited for *in situ* monitoring of air pollutants, and it is more efficient for testing water-soluble agents than for water-insoluble agents.

Tradescantia plant cuttings can be maintained in tapwater or nutrient solution and sustain normal life processes under artificial light. Thus, it is a best-suited material for studies of gaseous agents in glass chambers. Further study of the automobile exhaust fumes in the dilution chambers should continue.

Comparative studies of the clastogenic effects between diesel and gasoline engine emissions should be carried out using this bioassay, since the difference in clastogenicity of these two kinds of emissions is not yet well known at the present time.

Trad-MCN bioassay is ideal for testing clastogenic effects of pesticides either in the laboratory or in the field in the real-world situation.

Application of this bioassay to common beverages, foodstuff, pills and non-prescription drugs, and industrial and farm run-off in the real world is highly recommended.

SECTION 4

MATERIALS AND METHODS

GENERAL PROCEDURES

Greenhouse cultivated Tradescantia paludosa (Spiderwort) clone #03 was utilized in this study. Year-round growth and reproduction of this plant have been maintained by giving supplemented light during the short-day season of the year. Using the meiotic pollen mother cells in the inflorescences, a simple, quick short-term test called Tradescantia Micronucleus (Trad-MCN) bioassay was developed (11, 12) and extensively utilized in this investigation.

Within the 16 - 20 buds of an inflorescence, the younger buds usually contain pollen mother cells which are undergoing meiosis, and the pollen mother cells in one of these younger buds usually are in early prophase I stage. A large number of chromosome breaks can be induced in the stage because this meiotic stage is highly synchronized and their chromosomes are highly sensitive to mutagens (15, 18, 19, 21). Since the duration of the meiotic stages was known (22) (Fig. 1), broken chromosomes at early prophase can be observed in the later stages as fragments, laggards (Fig. 3), or micronuclei (Fig. 4) at a pre-determined time. Trad-MCN bioassay uses exclusively MCN in the well-synchronized tetrad stage (Fig. 5) as the end point. The duration of the recovery time (time needed for the early prophase I to progress to early tetrad stage) of this test system is around 24 hr after treatment (15). Treated samples, the inflorescences, ranging from 15 to 20 per experimental group were fixed in acteo-alcohol (1:3) after the recovery time and transferred into 70% ethanol after 24 hr of fixation. Generally, 5 - 9 microslides containing tetrads were prepared using the Aceto-carmine Squash method and scored randomly around 300 tetrads per slide. The means, standard deviations and standard errors were derived from 5 - 9 sample populations and used for statistical analysis. The "Standard Error of the Difference in Means" was used to determine the level of significance. The formula is shown below:

$$S_d = \sqrt{(SE_1)^2 + (SE_2)^2}$$

Where S_d is the value of the Standard Error of the Difference in Means. Level of significance at 0.05 or 0.01 is determined by the Difference in Means $(\bar{x}_1 - \bar{x}_2)$ divided by S_d.

SPECIAL TREATMENT PROCEDURES

In situ monitoring of air pollutants: The plant cuttings maintained in the water cups were exposed to the polluted atmosphere or carried on the roof top of an automobile which was parked in the public parking garage, or a bus or truck stop. A field control (FC) and home control (HC) were maintained (Fig. 6).

Gaseous treatment in chambers: The samples were set in the treatment chambers (Fig. 7) for a pre-determined duration. The concentration and flow rates were monitored with analytical and recording instruments during the course of treatment. Artifical light was used to illuminate the plants through the chamber to sustain the plant life. A remote fan was used in the air-tight chambers in order to stir up the air and the gaseous agent.

Treatment with water-soluble agents: The cut end of the stems of the plants were immersed in the solution (Fig. 8) containing a known concentration of the liquid agent for 6 - 24 hr. The treated stems were washed thoroughly after treatment and allowed to go through the recovery time. Sometimes, a new cut end was made if the original cut end was damaged during the treatment.

Treatment with water-insoluble agents: The insoluble agents were usually dissolved in DMSO or ETOH and diluted with water. Plant cuttings were maintained in the solution for treatment, usually for 6 hr. A blank control which contains the mixture of solvent and water alone in addition to a baseline control was maintained. Washing off the agents from the stem and cutting off the old cut ends were usually done after the treatment.

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SECTION 5

RESULTS AND DISCUSSION

Three types of air polluted environments were monitored: (a) Public Parking Garages, (b) Bus and Truck Stops, and (c) Industrial Sites, Farm and Laboratory Atmosphere. Results are given in Tables 1, 2, and 3.

Positive responses were obtained in each of these 3 conditions. The level of pollution in the public parking garages depends mainly upon the number of cars parked during the time of monitoring. The duration of exposure is not as important as the inclusion of the morning and evening rush hours. Outside weather conditions are not the main factor for pollution level in the garage since the parking garage is a partially enclosed structure.

The pollution level at the bus and truck stops depends mainly upon the weather condition. Only repeated monitoring under different weather conditions can give more meaningful data.

One industrial site, WRI, gave positive response in late fall. Repeated monitoring is needed to confirm the present finding. Fumes from paradichlorobenzene (PDB) in the herbarium gave inconclusive results after 6 hr exposure. Further tests under different conditions, such as frequencies of opening of the cabinet in which the herbarium sheets are stored, or heating and air-conditioning of the herbarium, should be considered in the future tests.

Three major components of the diesel engine emissions were tested: (a) Gaseous fumes, (b) Liquid state of the condensates, and (c) Pure gases found in the fumes. The results are given in Tables 4, 5, and 6.

Positive responses were obtained from relative high dosages (concentration of fumes combined with number of driving cycles). Further testing is in urgent need in order to establish a dose response curve. Comparative studies between diesel and gasoline engine exhaust fumes would be the most meaningful work at the present time since the relative clastogenicity of these two kinds of fumes is not yet clearly known (2).

The clastogenicity of the diesel emission condensates and the pure gases in the fumes need to be tested more carefully under this bioassay for the comparison with the results obtained from other bioassays, especially those obtained from mammalian systems (1, 3, 6, 7, 9).

These clastogenic effects from each and all the major components of automobile emissions seem to substantiate the findings of Blumer *et al* (5) on

incidence of cancer mortality among residents living near automobile highways.

Hydrazoic acid, a gaseous product of sodium azide under acid conditions, is highly clastogenic according to the results of the present studies. The doses expressed in Table 6 are, in reality, much lower than other pure gases used in this study because it was done by a single application of a known quantity of gas (ppm concentration was calculated on the basis of molrity and gas volume relationship) in an air-tight chamber rather than in a chamber with constant flow of a known concentration of gas. Aborted cells were observed in high dose treatment. This is a common phenomenon of over-dose in this bioassay system.

Sulfur dioxide which is known to be able to break chromosomes at 0.1 ppm level in Tradescantia pollen tubes (10) caused chromosome damage in pollen mother cells of the present bioassay at 1 ppm concentration after 22 hr of exposure.

Two kinds of agents in liquid form were tested in this study, namely water-soluble and water-insoluble forms. They were in the categories of food additives, heavy metallic compounds, vitamins, and known mutagen or carcinogens (3, 4, 6, 9, 16). Results of these studies are given in Tables 7 and 8. Ascorbic acid (AA) gave positive response when buffered with phosphate at the levels lower than the normal daily dose for humans, but not in unbuffered condition. Higher doses resulted in relative low MCN frequencies than lower doses of ascorbic acid and accompanied by the aborted cells. This is the typical indication of an over-dose (Table 7). Studies of Niacin were inconclusive. Sodium bisulfite (NBS) and Saccharin (SAC), widely used as food additives, showed positive response at relatively low dosages. High doses of these agents induced aborted cells in tetrads and microspores. It is very essential to carry out further studies on these agents.

Both inorganic chlorine compounds, $MnCl_2$ and $ZnCl_2$, caused chromosome damage. Further studies are needed to differentiate the effect of chlorine and metals.

Three water-insoluble agents were well known mutagens or carcinogens (1, 3, 6, 9, 16). They all gave positive responses under this bioassay. The negative response from benzo(a)pyrene (BaP) was obtained under the condition that insufficient solvent, DMSO, was used (Table 8). Therefore, little BaP was absorbed into the plant system because the agent was not fully dissolved in the treatment solution. Dimethyl sulfoxide (DMSO), a widely used solvent for water-insoluble agents to conduct mutagen tests in many other bioassays, was tested under this bioassay. Chromosome damage was noticeable at 10% solution of DMSO in water. Its synergistic effect when combined with other weak mutagens or even non-mutagens should be carefully studied. Physiological damage to the stem and leaves of Tradescantia plant cuttings was observed in higher concentration of DMSO.

The Spring Lake water (city reservoir) study is a year-round project. Relative lower MCN frequencies in lake water-treated groups than the control (chlorinated tapwater) might indicate a possible clastogenic effect of chlorine.

I was unable to obtain run-off water from power plants as originally proposed. Four of the original 12 chemicals proposed to be tested were substituted by their closely related compounds, Manganese chloride for cadmium bromide, lead acetate for lead chloride, Sodium bisulfite for potassium bisulfite and zinc chloride for zinc gluconate. Three chemicals were added for the 3 month extension period of this grant. They were sodium nitrite, ascorbic acid and hydrazoic acid.

In conclusion, the Trad-MCN bioassay is highly sensitive. A relatively lower level of significance (0.01) should be used to differentiate the positive from negative responses. The present series of studies reported here demonstrated the versatility of this bioassay. It is suitable for tests of gaseous and liquid agents and detect the effect of radiation. A typical 3-point dose-response test for chemicals can be conducted in 6 hr and results can be obtained in 24 - 48 hr. This bioassay is ideal for monitoring of air or water pollutants *in situ*. The fixed samples of a large-scale experiment can be stored away for months or years before the cytological observation and data collection. Tetrad and MCN preparations can be made into permanent slides for future references. Plant cuttings of *Tradescantia* can be used to serve as both *in vivo* and *in vitro* systems. They can sustain normal life processes in an enclosed chamber and tolerate relatively high acid or alkaline conditions as well as high toxicity. An over-dose situation can be observed through the signs of physiological damage. *Tradescantia* can be planted in the field along the common crops to test the effects of pesticides and conduct laboratory tests of the same pesticides (23, 24) to determine their mutagenic mechanism, synergism and metabolic pathways.

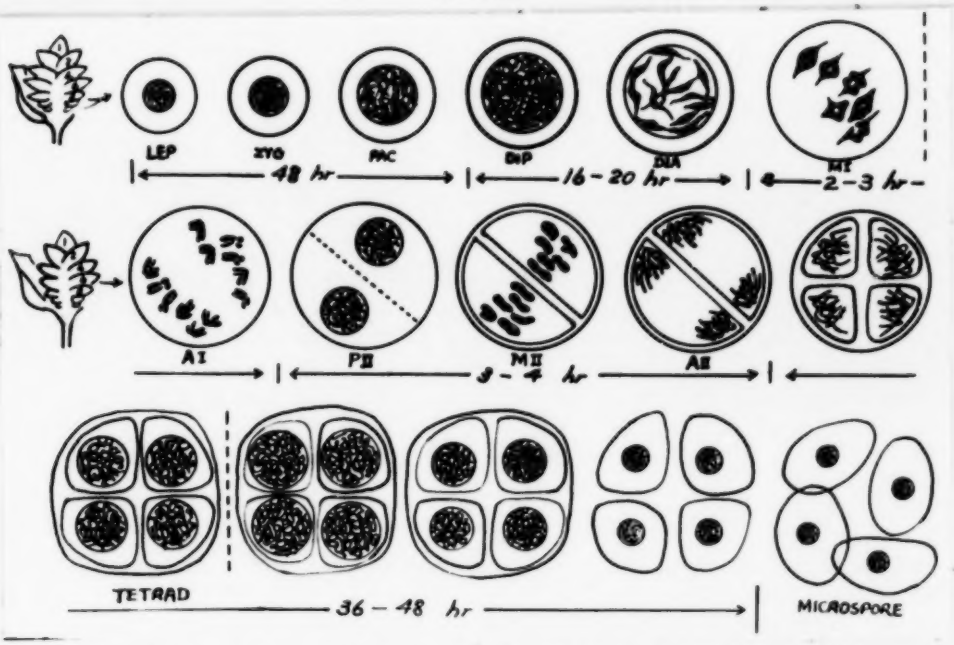

Fig. 1 Duration of the meiotic stages of Tradescantia pollen mother cells.

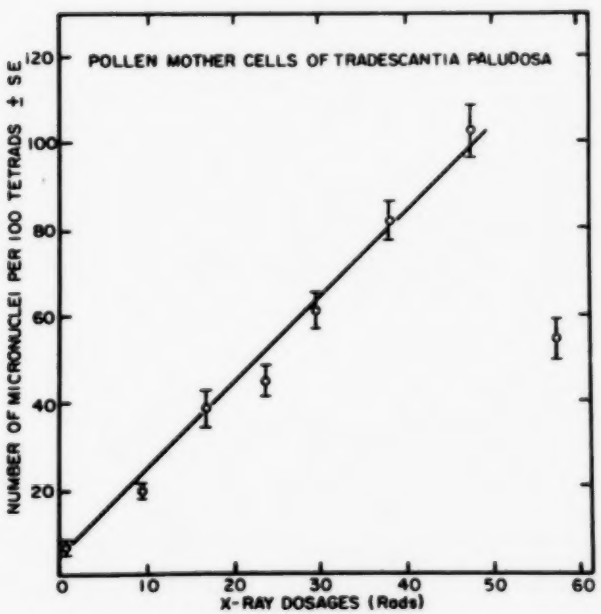

Fig. 2 Dose-response curve of X-ray treatment.

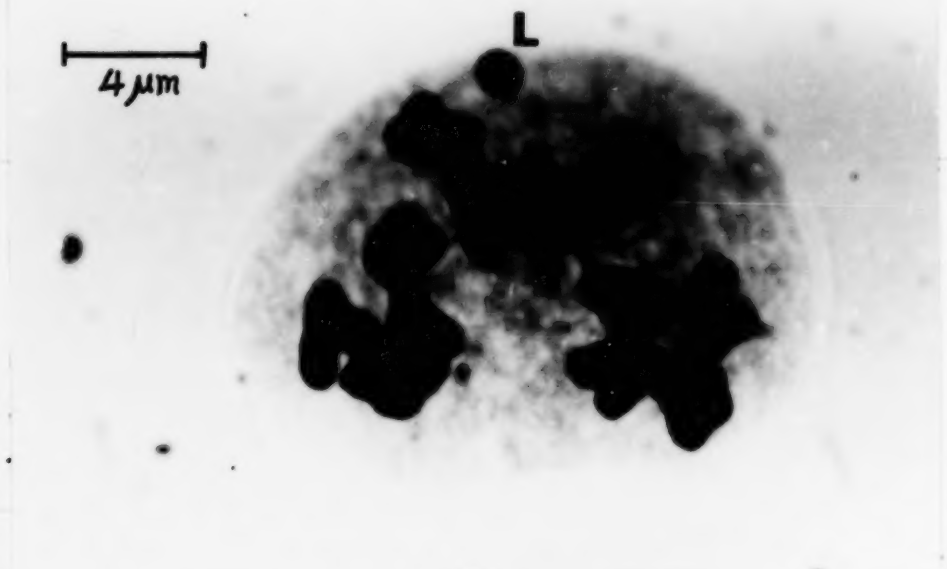

Fig. 3 Broken chromosome became laggard in Anaphase I of meiosis.

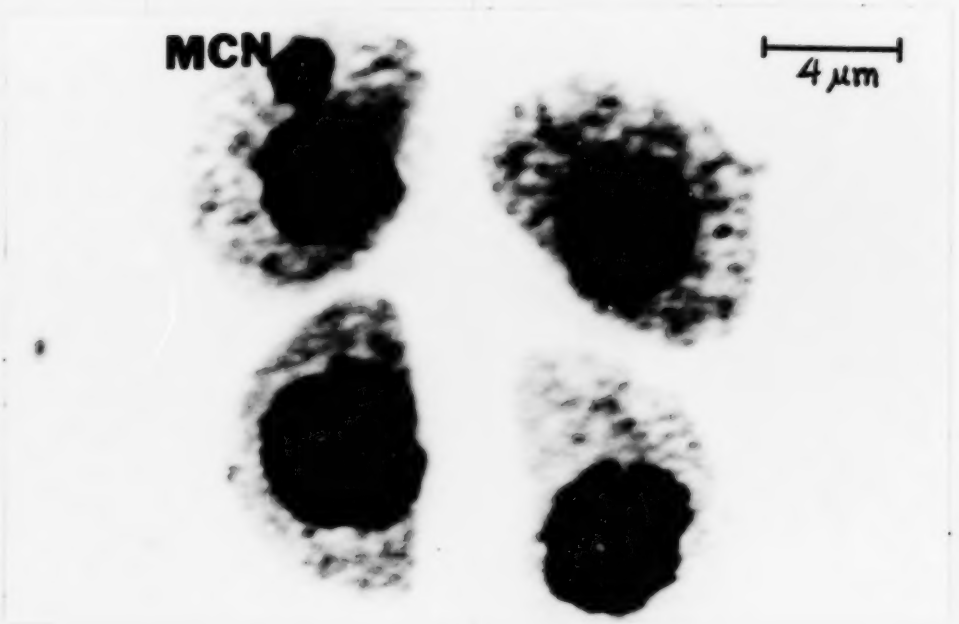

Fig. 4 Broken chromosome became a micronucleus in tetrad stage.

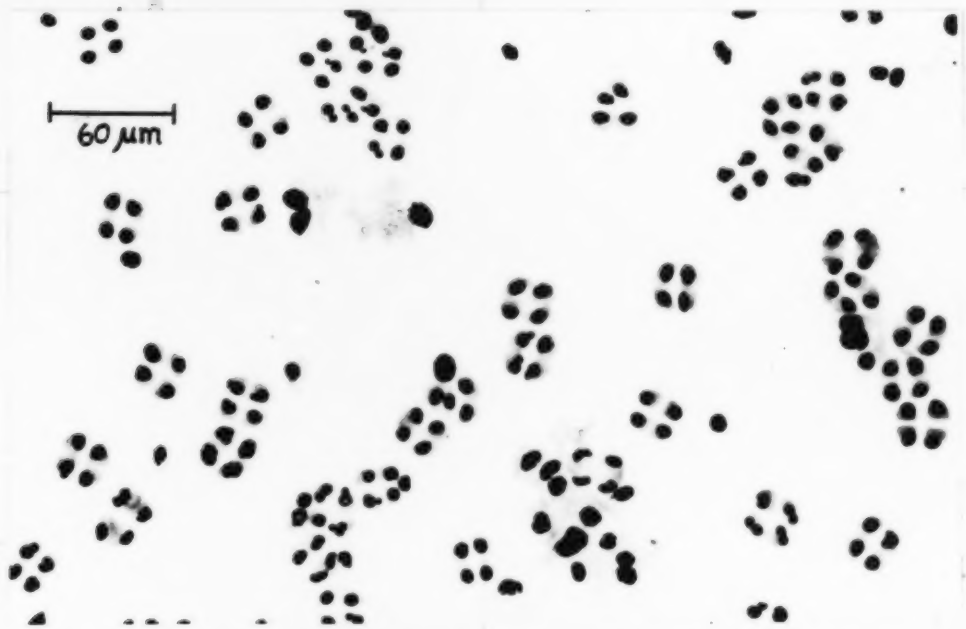

Fig. 5 A population of tetrads for scoring of the frequency of micronuclei.

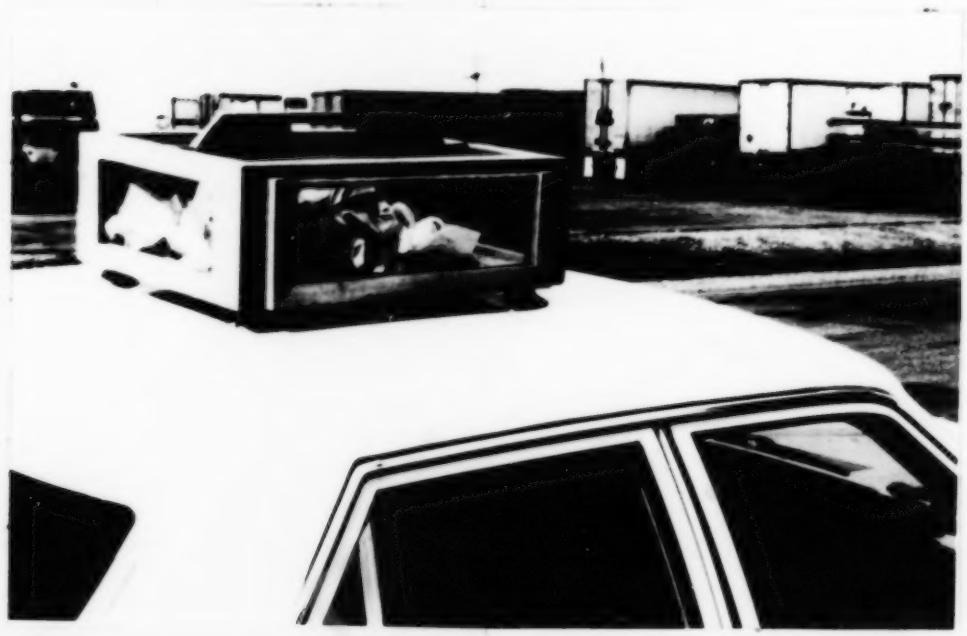

Fig. 6 In situ monitoring of air polluted sites, plant cuttings are carried in the screen cage.

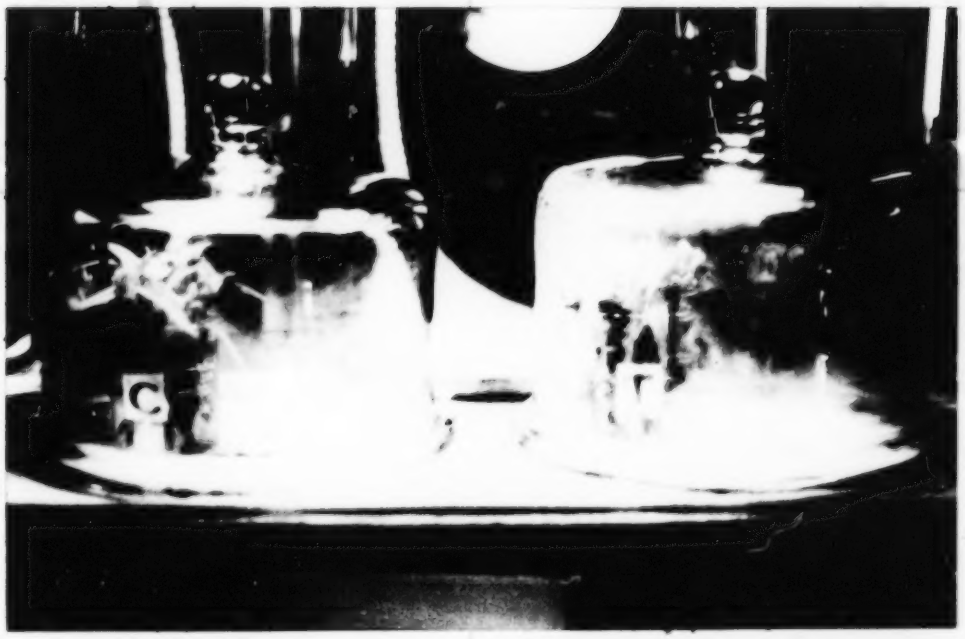

Fig. 7 Treatment of Tradescantia plant cuttings with gaseous agents.

Fig. 8 Treatment of Tradescantia plant cuttings with liquid agents.

By parking the automobile which carried the plant cuttings in a screen-cage on the rooftop in the public parking garages, the inflorescences were exposed to automobile exhaust fumes for 2 - 6 hr. Results are shown in Table 1 below:

TABLE 1: RESULTS OF IN SITU MONITORING OF PUBLIC PARKING GARAGES

Sites (code)	Expt. group	Dose (hr)	MCN/100 tetrads	SE	0.01 Signif.	Remarks
CMP-1	HC	0	6.65	1.60		
	FC	0	6.60	0.93		
	T-1	4	8.69	1.32	-	clear, wind from lake.
	T-2	6	8.34	0.85	-	clear, wind from lake.
DMP-1	HC	0	7.91	1.60		
	FC	0	9.00	2.00		
	T-1	1	9.90	1.00	-	
	T-2	2	11.00	1.70	-	
	T-3	3	10.00	1.60	-	
	T-4	4.5	12.30	2.50	-	
PSR-1	HC	0	7.80	1.18		
	FC	0	9.04	0.43		
	T-1	2	24.19	1.41	+	
	T-2	4	27.26	1.46	+	
	T-3	6	28.33	2.89	+	
PSR-2	HC	0	9.04	0.77		
	FC	0	10.50	1.34		
	T-1	2.5	16.28	2.73	+/-	few cars parked.
	T-2	4	12.20	1.07	+/-	few cars parked.

In situ monitoring was carried out near a bus stop on the city streets and and in the parking lot of truck stops. Inflorescences of the plant cuttings which were carried over the rooftop of the monitoring automobile were exposed to the diesel fumes and gasoline fumes from the exhaust systems of the vehicles. Results of 3 sites monitored are shown in Table 2 below:

TABLE 2: RESULTS OF IN SITU MONITORING OF THE BUS AND TRUCK STOPS

Sites (code)	Expt. group	Dose (hr)	MCN/100 tetrads	SE	0.01 Signif.	Remarks
DTS-1	HC	0	6.85	0.86		
	FC	0	7.81	1.07		
	T-1	2	10.13	3.44	-	
	T-2	4	6.29	0.56	-	
DTS-2	HC	0	9.70	2.26		
	FC	0	9.53	1.64		
	T-1	2	6.87	0.87	-	
	T-2	3	12.22	1.32	-	
PBS-1	HC	0	9.04	0.77		
	FC	0	10.50	1.34		
	T-1	0.5	9.73	0.85	-	
	T-2	1.0	13.53	1.57	+/-	
WTS-1	HC	0	5.12	0.69		
	FC	0	5.62	0.49		
	T-1	2.5	10.54	1.05	+	
	T-2	5.0	10.84	0.80	+	

In situ monitoring of air pollutants in the atmosphere of industrial sites, farm animal house as well as laboratory and office rooms, was carried out by exposing the plant cuttings on sites for 3 - 6 hr. Results are shown in Table 3 below:

TABLE 3: RESULTS OF IN SITU MONITORING OF INDUSTRIAL SITES, FARM AND LABORATORY ATMOSPHERE

Sites (code)	Expt. groups	Dose (hr)	MCN/100 tetrads	SE	0.01 Signif.	Remarks
GCI-1	HC	0	4.33	0.53		
	FC	0	7.70	1.19		
	T-1	month	9.12	1.20	+/-	clone #4430
WRI-1	T-1	3.5	15.89	2.45	+	clone #03
JTC-1	HC	0	7.04	1.28		
	T-1	4	9.70	1.41	-	cigarette smoke in office
PDB-1	HC	0	5.20	0.44		
	T-1	3	9.94	1.95	+/-	PDB fumes in herbarium
	T-2	6	10.34	1.27	+/-	PDB fumes in herbarium
UFO-1	HC	0	6.40	2.00		
	T-1	6	6.26	1.47	-	exhaust gas from swine house

Diesel exhaust fumes generated by the Nissan automobile at the HERL, US EPA, Research Triangle Park, NC were tested for clastogenicity with the Trad-MCN Bioassay. Exposure of the plant cuttings to 3 different concentrations of the fumes in combination of 1, 2, and 3 driving cycles (23 minutes per cycly)gave the results as shown in Table 4 below:

TABLE 4: RESULTS OF TRAD-MCN TESTS ON DIESEL EXHAUST FUMES

Experimental group (code number)		Concentration of gases* HC_e ppm	NO_x ppm	CO ppm	CO_2 %	Number driving cycles	MCN/100 tetrads (means)	SE	0.01 Signif. level
DEF - 1	BC						8.32	1.63	
	FC						5.67	0.61	
	T-1	3.04	1.5	0.9	0.04	1	5.45	0.68	-
	T-2	3.04	1.5	0.9	0.04	2	5.97	0.67	-
	T-3	3.04	1.5	0.9	0.04	3	7.75	0.78	-
DEF-2 & 3	BC						4.76	0.90	
	FC						5.04	0.70	
DEF - 2	T-1	46.95	18.0	18.0	0.49	1	8.56	2.07	-
	T-2	47.36	17.0	16.0	0.49	2	21.81	3.13	+
	T-3	43.84	18.0	17.0	0.49	3	10.90	1.68	-
DEF - 3	T-1	18.04	8.8	8.0	0.23	1	7.35	0.32	-
	T-2	20.65	10.0	9.5	0.25	2	7.54	1.62	-
	T-3	21.00	9.5	8.5	0.25	3	14.80	0.44	+
Positive control (40 R X-rays)							62.06	3.10	+

* Concentration of gases before 10 x dilution

Samples of diesel exhaust condensate were dissolved in DMSO or ETOH and diluted in water to obtain 10, 100, 150, and 200 ppm concentrations to treat plant cuttings through absorption and dialysis of the agent. Results of these treatments are shown in Table 5 below:

TABLE 5: RESULTS OF TRAD-MCN TESTS ON DIESEL EXHAUST CONDENSATE

Experimental groups		Concentrations (ppm)	Solvent used	MCN/100 tetrads	SE	0.01 Signif.	Remarks
DEC-1	BC	0	water	6.20	1.13		
	FC	0	water	3.70	0.16		
	C_d	0	DMSO	6.00	0.63		
	T_d-1	10	DMSO	7.10	1.74	-	
	T_e-1	10	ETOH	6.90	1.25	-	
	T_d-2	100	DMSO	7.05	0.63	-	
	T_e-2	100	ETOH	5.76	1.26	-	
DEC-2	BC	0	water	6.20	1.13		
	FC	0	water	4.92	0.18		
	C_e	0	ETOH	6.47	0.83		
	T_d-1	150	DMSO	10.91	2.10	-	
	T_e-1	150	ETOH	9.71	1.08	-	
	T_d-1	200	DMSO	10.20	1.25	-	
	T_e-2	200	ETOH	10.96	1.71	-	
Positive Control (40 R X-rays)				62.06	3.10	+	

Trad-MCN bioassay was applied to pure gases of NO_2, SO_2, O_3, and NO_2 combined with O_3 in the Northrop Services, Inc. facility. Hydrazoic acid generated through the reaction between sodium azide and hydrochloric acid was tested in the Laboratory of Environmental Mutagenesis of Western Illinois University. Results are shown in Table 6 below:

TABLE 6: RESULTS OF TRAD-MCN TESTS ON PURE GASES

Experimental groups		Concentration (ppm)	Duration (hr)	MCN/100 tetrads	SE	0.01 Signif.	Remarks
NO_2 - 1	C	0	0	5.76	0.44		
	T-1	5	6	4.56	0.53	-	
SO_2 -1	C	0	0	7.29	0.84		
	T-1	1	6	3.74	0.41	-	
O_3 - 1	C	0	0	5.80	0.44		
	T-1	5	5.5	4.20	0.95	-	
NO_2 - 2	C	0	0	3.68	1.11		
	T-1	5	24	7.53	0.23	+	
SO_2 - 2	C	0	0	6.17	0.66		
	T-1	1	22	10.01	1.03	+	
NO_2+O_3 - 1	C	0	0	6.18	0.66		
	T-1	5/each	3	5.60	0.60	-	
HN_3 -1	C	0	6	8.00			
	T-1	136*	6	21.20		+	
	T-2	272*	6	14.30		+	

*Single application in an air-tight chamber

Water soluble agents including vitamins, heavy metallic compounds, food additives were tested with Trad-MCN bioassay. All treatments were applied in 6 hr durations. Results are shown in Table 7 below:

TABLE 7: RESULTS OF TRAD-MCN TESTS ON WATER SOLUBLE AGENTS

Experimental groups		Concentration (% or mM)	MCN/100 tetrads	SE	0.01 Signif	Remarks
AA - 1	C	0	13.90	2.06		pH = 7.67
	T-1	0.0313%	27.64	1.36	+	Vitamin C tablets, buffered to pH = 6.6
	T-2	0.0625%	22.88	2.45	+/-	
	T-3	0.1250%	28.55	1.09	+	
	T-4	0.2500%	5.00	1.10	-	Aborted cells
	T-5	0.3750	12.00	1.20	-	Aborted cells
	T-6	0.7500	7.7	1.40	-	Aborted cells
AA - 3	C	0	5.53	1.49		pH = 7.51
	T-1	0.0156%	10.91	2.75	-	pH = 4.73, unbuffered
	T-2	0.3120%	7.62	1.15	-	pH = 3.85, unbuffered
	T-3	0.0625%	12.3	2.27	+/-	pH = 3.51, unbuffered
$MnCl_2$ - 1	C	0	3.73	0.62		
	T-1	1 mM	4.99	0.69	-	
	T-2	5 mM	5.32	0.74	-	
	T-3	10 mM	7.56	0.25	+	
	T-4	20 mM	7.34	1.23	+/-	
NBS - 1	C	0	5.12	0.84		
	T-1	0.1 mM	9.04	1.55	+/-	
	T-2	0.5 mM	7.58	0.64	+/-	
	T-3	1.0 mM	5.74	1.12	-	Over-dose, but no physiological damage observed

TABLE 7 (continued)

Experimental groups		Concentration (% or mM)	MCN/100 tetrads	SE	0.01 Signif.	Remarks
	T-4	10.0 mM	5.82	0.71	-	
NBS - 2	C	0	5.10	1.17		
	T-1	0.01 mM	7.50	1.25	-	
	T-2	0.05 mM	12.33	0.97	+	
NIC - 1	C	0	4.80	0.31		
	T-1	5 mM	5.92	0.51	-	
	T-2	10 mM	6.88	0.86	+/-	
NIC - 2	C	0	7.46	1.63		
	T-1	10 mM	6.10	0.94	-	
	T-2	15 mM	8.50	1.66	-	
	T-3	20 mM	10.00	3.37	-	
NNO - 1	C	0	6.79	2.03		
	T-1	0.5 mM	4.99	0.40	-	leaf tips turn yellow
	T-2	1.0 mM	6.13	1.38	-	leaf tips turn yellow
	T-3	5.0 mM	9.75	1.78	-	leaf tips turn yellow
	T-4	10.0 mM	6.56	0.47	-	leaf tips turn yellow
	T-5	50.0 mM	6.38	2.14	-	leaf tips turn yellow
PA - 1	C	0	7.20	0.77		pH = 6.67
	T-1	0.25%	10.70	2.38	-	pH = 2.84
	T-2	1.00	12.30	2.25	+/-	severe damage on leaves
PA - 2	C	0	5.29	0.66		
	T-1	0.0063%	8.44	1.53	-	unbuffered
	T-2	0.0125%	8.38	2.12	-	

TABLE 7 (continued)

Experimental groups		Concentration (% or mM)	MCN/100 tetrads	SE	0.01 Signif.	Remarks
	T-3	0.0250%	6.54	2.09	-	
	T-4	0.0500%	6.82	1.51	-	
	T-5	0.1000%	7.15	1.48	-	
PbAc - 1	C	0	5.73	0.66		
	T-1	1 mM	7.17	1.86	-	
	T-2	10 mM	8.30	0.96	+/-	
SAC - 1	C	0	4.74	0.44		
	C_s	0	4.33	0.35		
	T-1	0.25%	5.58	0.90	-	
	T-2	0.50%	6.72	0.58	+/-	
	T-3	1.00%	5.70	0.79	-	
SAC - 2	C	0	4.80	0.31		
	C_s	0	6.41	0.59		
	T-1	5 mM	8.82	0.94	+	
	T-2	25 mM	16.92	2.86	+	
	T-3	50 mM	10.48	0.92	+	
$ZnCl_2$ - 1	C	0	4.43	0.71		
	T-1	1 mM	10.68	1.71	+	
	T-2	10 mM	6.12	0.47	+/-	aborted cells, overdose
	T-3	50 mM	6.04	0.88	-	aborted cells, overdose

Water insoluble agents and their solvent, DMSO, plus Macomb city water supply (Spring Lake water) were tested with Trad-MCN bioassay. All treatments were administered in 6 hr duration except SLW. Results are shown in Table 8 below:

TABLE 8: RESULTS OF TRAD-MCN TESTS ON WATER INSOLUBLE AGENTS, THEIR SOLVENT, DMSO, AND WATER FROM MACOMB CITY RESERVOIR

Experimental groups		Concentration (% or mM)	Solvent used	MCN/100 tetrads	SE	0.01 Signif.	Remarks
BaP - 1	BC	0		8.21	1.74		
	C_d	0	DMSO	8.66	2.04		
	T-1	0.1 mM	DMSO	8.06	0.85	-	2.5% DMSO, BaP fully dissolved
	T-2	0.5 mM	DMSO	8.26	1.26	-	
BaP - 2	BC	0		5.10	1.17		
	C_d	0	DMSO	7.97	0.62		
	T-1	0.05 mM	DMSO	15.50	1.52	+	10% DMSO, BaP fully dissolved
	T-2	0.10 mM	DMSO	23.13	3.78	+	
CHA -1	C	0		8.50	0.68		
	T-1	0.25%		15.00	1.70	+	unbuffered
	T-2	0.50%		56.5	3.50	+	
MH -1	C	0		8.51	0.69		
	T-1	8.90 mM		15.34		+	unbuffered
	T-2	11.20 mM		17.23		+	unbuffered
	T-3	13.40 mM		18.15		+	unbuffered
	T-4	15.60 mM		31.35		+	unbuffered
	T-5	17.90 mM		17.84		+	physiological damage
DMSO - 1	C	0		9.10	0.95		
	T-1	5%		7.34	0.61	-	

TABLE 8 (continued)

Experimental groups		Concentration (% or mM)	Solvent used	MCN/100 tetrads	SE	0.01 Signif.	Remarks
	T-2	10%		13.08	1.63	+/-	
	T-3	20%		10.26	1.17	-	physiological damage
DMSO -2	C	0		6.25	0.73		
	T-1	20%		11.30	1.12	+	
	T-2	30%		15.16	1.80	+	physiological damage
	T-3	40%		5.93	1.06	-	severe damage
SLW - 1	C			4.80	0.31		chlorinated water
	T-1			4.30	0.42	-	
	C			7.46	1.63		chlorinated water
	T-1			5.55	0.27	-	

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